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R. J. O. Woods
P. Z. Sobocinski
W. J. Canterbury
N. S. Mathewson
K. M. Hartley

ARMED FORCES RADIOBIOLOGY RESEARCH INSTITUTE
Defense Nuclear Agency
Bethesda, Maryland

Research was conducted according to the principles enunciated in the "Guide for Laboratory Animal Facilities and Care," prepared by the National Academy of Sciences - National Research Council.

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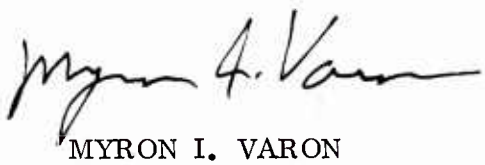
R. J. O. WOODS
P. Z. SOBOCINSKI
W. J. CANTERBURY
N. S. MATHEWSON
K. M. HARTLEY

JOE E. WEST
Colonel, USAF, VC
Chairman
Radiation Biology Department



MYRON I. VARON
Captain MC USN
Director

ARMED FORCES RADIOBIOLOGY RESEARCH INSTITUTE
Defense Nuclear Agency
Bethesda, Maryland

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ABSTRACT

This study was performed to determine whether alloxan treatment of rats alters levels of the terminal carbohydrate residues L-fucose and sialic acid of serum glycoproteins. Results indicate that in the uncompensated diabetic animal a chronic depression of serum sialic acid level occurs with no apparent alteration in the level of L-fucose. The depression in sialic acid level may be attributed in part to decreased activities of hepatic enzymes involved in sialic acid synthesis similar to those observed by others after treatment of rats with the diabetogenic agent streptozotocin. The lack of any significant alteration in the level of L-fucose fails to confirm, in the experimental diabetic animal, the increased protein-bound fucose levels reported in human diabetics. Administration of insulin was not effective in modifying the sialic acid response after alloxan treatment.

I. INTRODUCTION

Numerous studies, cited by McMillan,[10] indicate that alterations occur in the serum levels of various protein-bound carbohydrates (neutral hexoses, L-fucose, and sialic acid) in human diabetes. There is, however, a lack of unanimity when the direction and magnitude of some of these alterations are considered. For example, serum L-fucose levels have been reported to be elevated[10,11,15] and unchanged[5,8,14] in human diabetics when compared to levels observed in controls. Nonspecificity of analytical methods[8,12] and differences in subject selection[10] appear to have contributed to these contradictory findings. In addition, it is unclear whether the reported elevations cited above are modified by controlling the diabetic state.

In contrast to the relatively large number of serum glycoprotein-carbohydrate studies performed with human diabetics, only a few studies have been performed with the experimentally induced diabetic animal. These latter studies have been limited to hexosamine metabolism.[10,16,20] Neither the synthesis of hexosamine from glucose[20] nor serum protein-bound hexosamine levels[16] appears to be altered in the alloxanized rat.

The present study was performed to determine whether alloxan-induced alterations occur in the levels of protein-bound L-fucose and sialic acid and if so whether the administration of insulin modifies the observed response to alloxan. The results reported below indicate that alloxan treatment chronically depresses serum sialic acid levels with no apparent alteration in L-fucose levels. The selective depression of one terminal residue in the absence of altered hexosamine metabolism suggests that the site(s) of altered carbohydrate metabolism in the alloxanized rat occurs in the enzymic

systems involved in the biosynthetic pathway of sialic acid similar to those reported for the diabetogenic agent streptozotocin.[7]

II. MATERIALS AND METHODS

Male Sprague-Dawley rats, weighing 200-220 g, were fed Wayne Lab Blox* and tap water ad libitum. Diabetes was induced in randomly selected rats following a 24-hour fast by a single subcutaneous injection of a 10 percent solution (w/v) of alloxan monohydrate† in physiological saline, 100 mg/kg body weight. Control animals were given equal volumes of saline. Animals were decapitated at 3- to 14-day intervals after alloxan injection. Blood was collected from the neck vessels, allowed to clot, and the serum separated by centrifugation for the analytical determinations described below. Serum samples were either analyzed on the day of collection or after storage at -60°C for no more than 1 week. Serum was analyzed for glucose by an automated method employing glucose oxidase.[18] Sialic acid (as N-acetylneuraminic acid) was determined by an automated method[9] employing neuraminidase from Clostridium perfringens‡ and the thiobarbituric acid reaction of Warren.[21] L-fucose was assayed by the following three methods: (1) the manual method of Winzler,[22] (2) a modification of Winzler's method employing a correction for interfering substances,[4] and (3) by borate ion-exchange chromatography.[19] Total protein and protein-bound hexose levels were determined by methods previously described.[4]

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* Allied Mills, Inc., Chicago, Illinois
† Alloxan, Eastman Organic Chemicals, Rochester, New York
‡ Sigma Chemical Company, St. Louis, Missouri

Only diabetic rats having serum glucose levels ≥500 mg/dl were used in determining the levels of serum constituents after alloxanization. In experiments performed with compensated diabetics, zinc insulin suspension* was administered (intraperitoneally) daily for 6 days, 2 units/kg body weight, beginning on the 4th day after alloxan injection.

Statistical comparisons were made using Student's "t" test and the Behrens-Fisher modified t when applicable.[1] P values ≤0.01 were considered significant.

III. RESULTS

The time-course of alterations in serum sialic acid levels in uncompensated diabetic and control rats during the experimental period, 3 to 15 days postalloxanization, is shown in Figure 1. Administration of alloxan induced a significant depression of sialic acid levels during the entire observation period. In a separate study, reduced serum sialic acid levels were also observed in alloxanized animals which were allowed to survive untreated for the development of diabetic cataracts, approximately 5 months after alloxan treatment.

The interval 10 to 14 days postalloxanization was selected for the study of L-fucose, neutral hexose, and total protein levels. The results of this study are shown in Table I. No significant differences were observed when L-fucose levels in diabetic and control rats were compared. This finding was confirmed when borate ion-exchange chromatography was performed on pooled sera from diabetic and control animals (results not shown). We did, however, observe a significant depression of serum protein-bound hexose levels in diabetic rats 12 and 13 days postalloxanization and a

* Insulin, Eli Lilly and Company, Indianapolis, Indiana

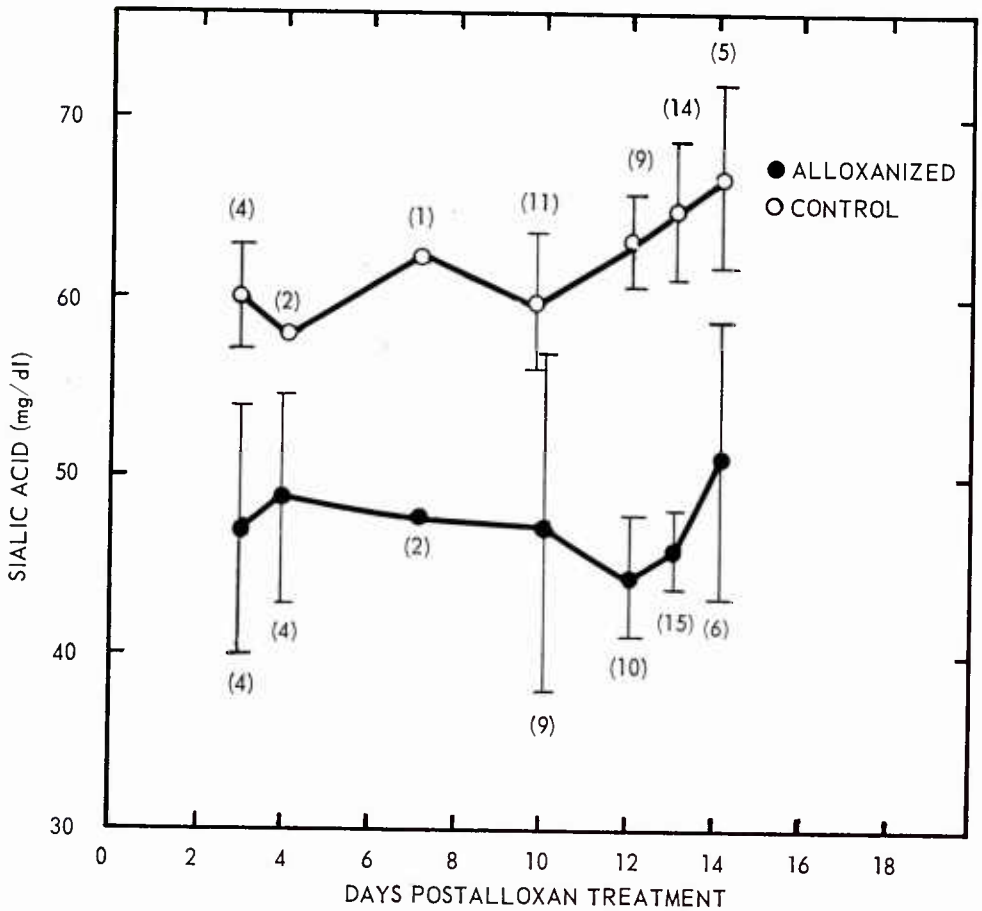

Figure 1. Time-course of alterations in serum sialic acid levels in alloxanized and control rats. Alloxan treatment occurred on day 0. Values shown are the mean ± S.D. except the mean only is shown when one or two animals were used. Number of animals is shown in parentheses. When sample sizes greater than two were compared, diabetic and control values were significantly different ($P \leq 0.01$) at each experimental period.

consistently lower mean value for the diabetic rats during the 10- to 14-day interval when compared to the values obtained for control animals. No significant differences were observed when total protein values were compared.

Twelve animals that received daily insulin injections had a mean serum sialic acid concentration of 47.1 mg/dl ± 3.4 (S.D.) at the time of euthanasia. This level is not significantly different from that obtained for animals not administered insulin.

Table I. Serum L-Fucose, Protein-Bound Hexose, and Total Protein Levels in Alloxan Diabetic and Control Rats at Various Time Intervals After Treatment

Treatment*	Time†	L-fucose‡§		Protein-bound hexose‡	Total protein**
		(A)	(B)		
Alloxan (9)	10	4.5 ± 0.8	8.6 ± 0.6	162.2 ± 21.0	54.2 ± 6.7
Control (11)	10	4.1 ± 1.4	8.1 ± 1.2	172.0 ± 10.0	56.6 ± 3.1
Alloxan (10)	12	4.3 ± 1.0	8.4 ± 0.9	157.1 ± 9.6††	56.2 ± 11.3
Control (9)		3.1 ± 1.4	7.6 ± 1.5	177.0 ± 10.8	57.2 ± 3.6
Alloxan (15)	13	5.4 ± 0.9	9.1 ± 1.0	143.3 ± 7.8††	53.3 ± 6.1
Control (14)		5.9 ± 1.0	9.1 ± 1.0	167.0 ± 9.0	51.5 ± 2.1
Alloxan (6)	14	4.8 ± 0.6	9.0 ± 0.6	164.3 ± 17.3	54.6 ± 6.3
Control (5)		4.7 ± 0.4	9.0 ± 0.4	172.0 ± 22.9	62.3 ± 3.0

* Alloxanized animals received a single subcutaneous injection of alloxan monohydrate in saline, 100 mg/kg body weight. Control animals received an equal volume of saline. Number of animals is shown in parentheses.

† Time refers to days after treatment

‡ Concentration expressed as mg per dl, mean ± S. D.

§ Values determined by an analytical method with (A) and without (B) correction for interfering hexoses, see Methods section

** Concentration in terms of mg per ml, mean ± S. D.

†† Significant, $P \leq 0.01$

IV. DISCUSSION

Results obtained in this study indicate that in the uncompensated alloxan-induced diabetic rat a marked and sustained depression in the serum concentration of protein-bound sialic acid occurs without any significant alteration in protein-bound L-fucose levels. Because of the nonspecificity of the commonly employed Dische-Shettles method for fucose determinations,[17] the findings for fucose levels in this study were confirmed by borate ion-exchange chromatography. The method used to measure

sialic acid levels employs enzymic cleavage of α-ketosidically bound sialic acid residues. Therefore, the sialic acid levels reported in this study should be interpreted as neuraminidase-labile sialic acids. We do not have information at the present time on the occurrence or extent of neuraminidase-resistant linkages in serum glycoproteins of our experimental animals.

The selective depression of one terminal residue of serum glycoproteins suggests that the metabolic alteration responsible for these observations occurs at some biosynthetic or catabolic site specific for the sialic acid residue. Such a site has recently been described by Maley et al.[7] These investigators, employing the diabetogenic agent streptozotocin, in rats, reported decreased activities of several enzymes involved in hepatic synthesis of sialic acid. It seems likely that our results may be explained by a similar enzymic defect induced by alloxan; however, this remains to be determined.

Studies performed with human diabetics indicate that serum sialic acid levels are either unchanged[3,5] or elevated[2] when compared to control values. Although our observations are not in agreement with those for human diabetics, it is apparent that the degree of insulin deficiency in our experimental animals was severe. Therefore, our results may not be comparable to the studies cited above. It is not known whether the depression in sialic acid synthesizing enzymes observed after streptozotocin treatment[7] reflects (1) a toxic effect on the enzyme systems by the diabetogenic agent, (2) a phenomenon of the insulin-deficient state, or (3) a combination of 1 and 2. We could not, however, demonstrate that insulin treatment modifies the alloxan-induced serum sialic acid response.

Data obtained for L-fucose levels indicate that there is no alteration in the alloxan-induced diabetic animal. This finding does not provide evidence to support the altered carbohydrate metabolism leading to enhanced fucose production postulated by Shaw et al.[15] and McMillan[11] to occur in the human diabetic state. Our results appear to support the findings of Howard and Kelleher[5] and Saifer and Weintraub[14] of unaltered fucose metabolism in the diabetic state. It seems likely that part of the discrepancy in experimental evidence relative to L-fucose levels in human diabetics could be explained by the concomitant presence of other disease processes known to disturb glycoprotein metabolism.[6]

The administration of alloxan to rats has been reported to either selectively elevate or depress the levels of various specific serum glycoproteins[16] and to depress protein-synthetic activity of membrane-bound hepatic ribosomes.[13] We believe that the lowered protein-bound hexose levels observed in diabetic rats in this study may be attributed in part to the altered hepatic glycoprotein metabolism induced by alloxan since measurement of the serum levels of protein-bound carbohydrates is an average estimate of a very heterogeneous molecular population. Increased or decreased hepatic synthesis of any number of specific glycoproteins with marked variation in bound carbohydrate composition would be expected to influence the observed serum levels. A supplementary metabolic defect, i.e., hepatic sialic acid synthesis, could further depress serum sialic acid levels.

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13. ABSTRACT

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